Potential neuroprotective benefits of erythropoietin during experimental hypothermic circulatory arrest

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Objective: Recent studies have shown that erythropoietin protects neurons from glutamate toxicity and ischemia. This study was performed to evaluate the potential neuroprotective effect of erythropoietin during experimental hypothermic circulatory arrest.

Methods: Twenty pigs were randomized to receive intravenously either 500 IU/kg recombinant human erythropoietin or saline before a 75-minute period of hypothermic circulatory arrest at an intracerebral temperature of 18°C.

Results: After the administration of erythropoietin, its concentration in the cerebrospinal fluid increased 4.5-fold 8 hours after the start of rewarming, whereas it did not increase in control animals. The 7-day survival rate was 60% in the erythropoietin group and 70% in the control group ($P = 1.0$). No significant differences were observed between the study groups in terms of electroencephalography, behavioral score, and histopathologic score. The erythropoietin group had higher vascular resistance and mean arterial pressure values, lower intracerebral concentrations of glutamate and glycerol, higher brain tissue oxygen tension, and lower apoptotic index.

Conclusions: Administration of 500 IU/kg erythropoietin intravenously before hypothermic circulatory arrest was followed by an increased erythropoietin concentration in the cerebrospinal fluid. Although previous studies have demonstrated neuroprotective effects of erythropoietin during brain ischemia, the present study, using a chronic porcine model, failed to show any significant benefit after administration of erythropoietin in terms of mortality or brain histopathology. Lower intracerebral concentrations of glutamate and glycerol, higher brain tissue oxygen tension, and lower apoptotic index observed in the erythropoietin group, however, suggest that a distinct neuroprotective effect of erythropoietin might be achieved at different dosages and timing of administration.

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Erythropoietin (EPO) is a glycoprotein first characterized as a hematopoietic growth factor produced by the kidney. Recent studies have shown that astrocytes also produce EPO in a hypoxia-inducible manner, and that EPO receptors are expressed in neurons.[1-4] There is evidence that EPO protects primary cultured neurons from *N*-methyl-D-aspartate receptor–mediated glutamate toxicity,[2] and that intracerebroventricular[3,5-7] and systemic administration[1,7,8] of recombinant human EPO (r-Hu-EPO) reduces neuronal ischemic injury. Because these in vivo and in vitro

stroke model studies have provided convincing evidence on the neuroprotective effect of systemically administered EPO, we planned the present study to evaluate its potential neuroprotective efficacy in a chronic porcine model of hypothermic circulatory arrest (HCA).

Materials and Methods

Twenty female juvenile pigs (age, 8-10 weeks) of a native stock were randomly assigned to receive either 500 IU/kg r-Hu-EPO or placebo as an intravenous injection before a 75-minute period of HCA at an intracerebral temperature of 18°C.

Preoperative Management

All animals received humane care in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996. The study was approved by the Research Animal Care and Use Committee of the University of Oulu.

Anesthesia and Hemodynamic Monitoring

Anesthesia was induced with ketamine hydrochloride (10 mg/kg administered intramuscularly) and midazolam (1 mg/kg administered intramuscularly). A peripheral venous catheter was inserted into the right ear for administration of drugs and to maintain fluid balance with Ringer acetate. Anesthesia was deepened with thiopental sodium (125-250 mg administered intravenously), and pancuronium bromide (4 mg administered intravenously) was given for muscular paralysis. Cefuroxime, 1.5 g, was given as antibiotic prophylaxis at anesthesia induction, 8 hours after the start of rewarming, and before extubation.

After endotracheal intubation, the animals were maintained on positive-pressure ventilation with 50% oxygen, and anesthesia was maintained with isoflurane (1.2%-1.3%). Electrocardiographic monitoring was started. An arterial catheter was positioned into the left femoral artery for arterial pressure monitoring and blood sampling. A thermodilution catheter (CritiCath, 7F; Ohmeda GmbH & Co, Erlangen, Germany) was placed through the left femoral vein to allow blood collecting, pressure monitoring in the pulmonary artery, and recording of blood temperature and cardiac output. A 10F catheter was placed in the urinary bladder for monitoring urine output. Blood, rectal, esophageal, epidural, and intracerebral temperatures were monitored continuously.

Brain Microdialysis and Intracerebral Monitoring

A temperature probe was placed into the epidural space through a cranial hole made in the left side anteriorly to the coronal suture. A catheter for the measurement of intracerebral tissue oxygen partial pressure (Revodoxe Brain Oxygen Catheter-Micro-Probe, REF. CC1.SB; GMS, Mielkendorf, Germany) was inserted through a hole located at the right side anteriorly to the coronal suture. Another temperature probe (Thermocouple Temperature Catheter-Micro-Probe, REF. C8.B, GMS) for the measurement of intracerebral temperature, together with an intracranial pressure-monitoring catheter (Codman Micro-Sensor ICP Transducer; Codman & Shurtleff, Inc, Raynham, Mass), were placed through a hole located at the left side posteriorly to the coronal suture. Intracerebral temperature and brain tissue oxygen partial pressure were monitored with the Licox CMP Monitor (GMS). Intracranial pressure was monitored with the Codman ICP Express Monitor (Codman & Shurtleff, Inc).

The microdialysis catheter (CMA 70; CMA/Microdialysis, Stockholm, Sweden) was placed into the brain cortex to a depth of 15 mm below the dura mater through a hole located at the right side posteriorly to the coronal suture. The catheter was connected to a 2.5-mL syringe placed into a microinfusion pump (CMA 106, CMA/Microdialysis) and perfused with Ringer solution (Perfusion Fluid CNS, CMA/Microdialysis). Samples were collected at different time points. The concentrations of cerebral tissue glucose, lactate, glutamate, and glycerol were measured immediately after collection with a microdialysis analyzer (CMA 600, CMA/Microdialysis) by using ordinary enzymatic methods.

Electroencephalographic Monitoring

Cortical electrical activity was registered by 4 stainless-steel screw electrodes of 5 mm in diameter implanted into the skull over the parietal and frontal areas of the cortex by using a digital electroencephalography recorder (Nervus, Reykjavik, Iceland) and an amplifier (Magnus EEG 32/8, Reykjavik, Iceland). Sampling frequency was 256 Hz, and bandwidth was 1.6 to 70 Hz. All 4 electroencephalographic electrodes were referenced to another screw electrode, which, together with a ground screw electrode, was implanted over the frontal sinuses.

The isoflurane level was adjusted so that electroencephalography showed a steady burst-suppression pattern. Then isoflurane end-tidal concentration was kept at this steady level until the end of monitoring. An electroencephalogram was recorded for 10 minutes before the injection of r-Hu-EPO/saline to make the first baseline recording of steady burst-suppression activity and for another 10 minutes between the injection and the cooling period to make the second baseline recording. After HCA, electroencephalographic recording was restarted and continued until extubation. The duration of bursts were measured from 5-minute electroencephalographic samples at 1-hour intervals with a custom-made automatic analysis program. Artifact periods were excluded from each 5-minute sample, and after that, the sum of burst durations was counted as a percentage of the sum of artifact-free electroencephalography in this sample. This percentage was used as a measure of electroencephalographic activity in the analysis.

Cardiopulmonary Bypass

Through a right thoracotomy in the fourth intercostal space, the right thoracic vessels were ligated, the pericardium was opened, and the heart and great vessels were exposed. A membrane oxygenator (Midiflow D 705; Dideco, Mirandola, Italy) was primed with 1 L of Ringer acetate and heparin (5000 IU). After systemic heparinization (500 IU/kg), the ascending aorta was cannulated with a 16F arterial cannula, and the right atrial appendage was cannulated with a single 24F atrial cannula. Nonpulsatile cardiopulmonary bypass (CPB) was initiated with 100% oxygen (maintained during cooling and rewarming periods) at a flow rate of 100 $mL \cdot kg^{-1} \cdot min^{-1}$, and the flow was adjusted to maintain a perfusion pressure of 50 mm Hg. A 12F intracardiac sump cannula

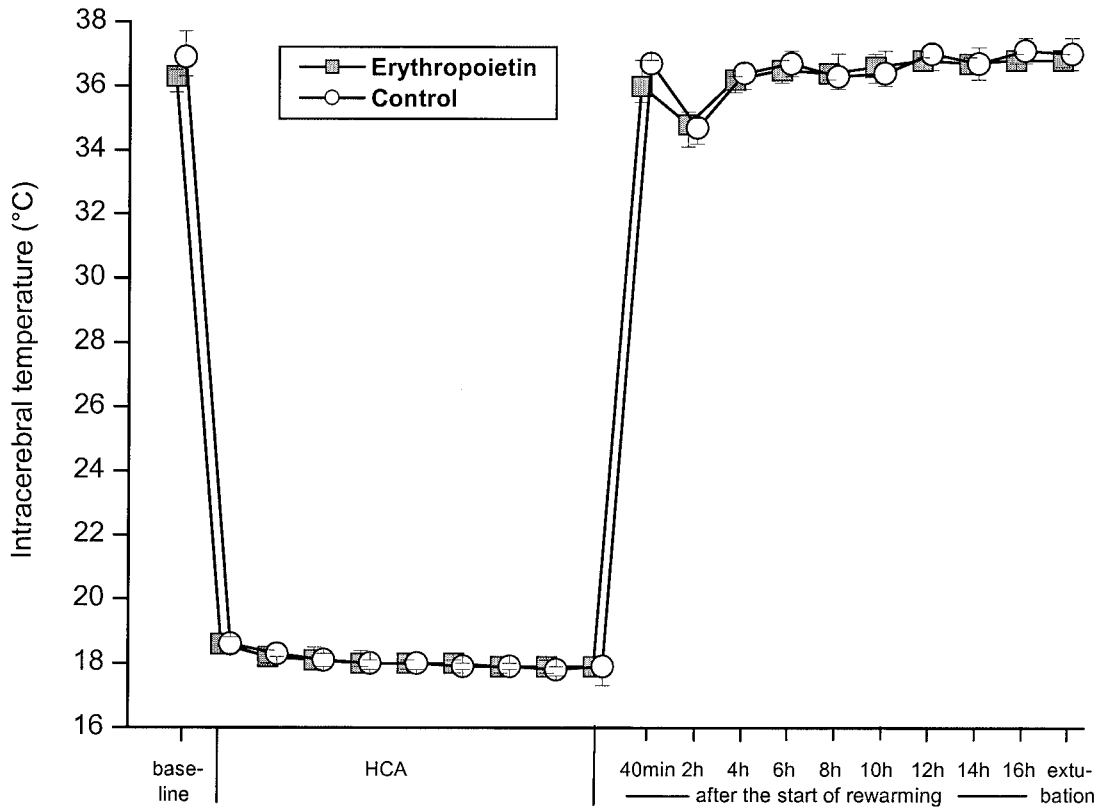

Figure 1. Intracerebral temperatures in pigs during the experimental protocol. Values are expressed as medians with interquartile ranges (25th-75th percentile).

was positioned in the left ventricle through the apex of the heart for decompression of the left side of the heart during CPB. A heat exchanger was used for core cooling. The pH was maintained with alpha-stat principles at 7.40 ± 0.05, with an arterial carbon dioxide tension of 4.0 to 5.0 kPa uncorrected for temperature.

Drug Administration

Recombinant human EPO is a human 165-amino-acid glycoprotein with a molecular weight of 32 to 40 kd. It is manufactured by using recombinant DNA technology. It contains the identical amino acid sequence and has the same biologic activity as isolated natural EPO.[9] The r-Hu-EPO used was epoetin β (Neo-Recormon; Roche Co, Basel, Switzerland), which was formulated as a sterile, colorless liquid in distilled water (100,000 IU [830 μg]/5 mL). The drug dosage was 500 IU/kg. Before the injection, the drug was diluted with isotonic sodium chloride to obtain a total volume of 5 mL. The placebo consisted of a corresponding volume of isotonic sodium chloride. A slow 5-mL intravenous injection was given in a double-blinded fashion according to the randomization protocol.

Experimental Protocol

After baseline measurements and 60 minutes before the start of cooling perfusion, the r-Hu-EPO/placebo was given to the animals. A second baseline electroencephalogram was recorded after that, and the cannulation of the heart and aorta was done. A cooling period of 60 minutes was carried out to attain an intracerebral temperature of 18°C. Then a 75-minute period of HCA was started. The ascending aorta was crossclamped just distal to the aortic cannula, and cardiac arrest was induced by injecting potassium chloride (3 g) through the aortic cannula. Topical cardiac cooling with ice slush was begun and maintained throughout the HCA period. During HCA, the epidural and intracerebral temperatures were maintained at a level of 18°C with ice packs placed over the head. After 75 minutes of HCA, rewarming was started. The animals were rewarmed to a core temperature of 37°C during 60 minutes of reperfusion, and this temperature was maintained until the end of the experiment. The temperatures were regulated with heat-exchanger mattresses, heating lamps, and ice packs.

During rewarming, the left ventricular sump cannula was removed, and furosemide (40 mg), mannitol (15 g), methylprednisolone (80 mg), lidocaine (40-160 mg), and calcium glubionate (1375 mg) were administered. After weaning from CPB, cardiac support was provided with dopamine. The animals were kept in isoflurane anesthesia, and they were ventilated with 100% oxygen until 2 hours after the start of rewarming and after that with 50% oxygen until the following morning (18 hours after the start of rewarming), when they were extubated and moved to a recovery room.

During the experiment, hemodynamic and metabolic measurements (pulse rate, systemic and pulmonary arterial pressures, central venous pressure, pulmonary capillary wedge pressure, cardiac output, intracranial pressure, intracerebral tissue oxygen partial

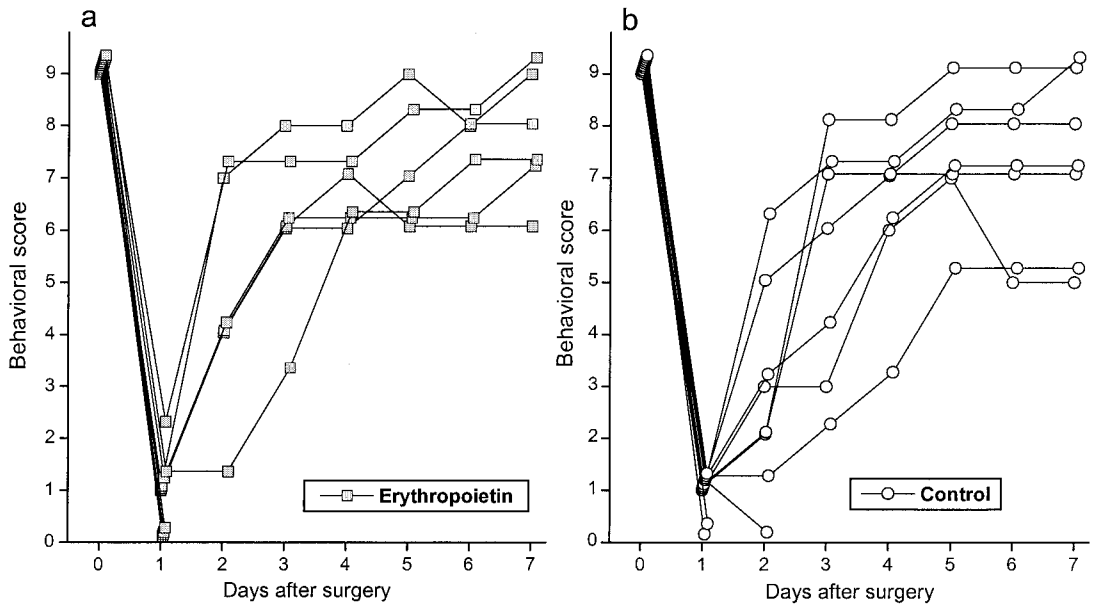

Figure 2. Daily score indicating behavioral recovery after 75 minutes of HCA. A score of 9 indicates essentially complete recovery.

pressure, temperatures, arterial and venous pH, oxygen and carbon dioxide partial pressures, oxygen saturation, oxygen concentration, hematocrit, hemoglobin, sodium, potassium, and glucose [Ciba-Corning 288 Blood Gas System; Ciba-Corning Diagnostic Corp, Medfield, Mass]; lactate [YSI 1500 analyzer; Yellow Springs Instrument Co, Yellow Springs, Ohio]; leukocyte differential count [Cell-Dyn analyzer; Abbot, Santa Clara, Calif]; and creatine kinase [CK] and its isoenzymes [CK-MM, CK-MB, CK-BB, Hydrasys LC-electrophoresis, Hyrys-densitometry; Sebia, France]) were recorded continuously or at baseline; at the end of cooling (immediately before institution of HCA); at 40 minutes, 2 hours, 4 hours, and 8 hours after the start of rewarming; and before extubation.

EPO Assay

EPO concentrations were measured from samples of plasma collected before drug administration (baseline), at the end of cooling, 40 minutes and 8 hours after the start of rewarming, and at extubation. EPO concentrations in the cerebrospinal fluid were measured after collecting the liquid from the lumbar cerebrospinal space before drug administration, 8 hours after the start of rewarming, and at extubation. Only those samples free from blood were accepted for the analysis.

EPO concentrations were determined by using a commercially available bioassay (2nd IRP EPO, human, for bioassay NIBSC Code: 67/343) by Medix Laboratories (Espoo, Finland). The lower limit of detection was 8.0 U/L.

Postoperative Evaluation

Postoperatively, all the animals were evaluated daily by an experienced observer who was blinded to the study group by using a species-specific quantitative behavioral score. The assessment quantified mental status (0 = comatose, 1 = stuporous, 2 = depressed, and 3 = normal), appetite (0 = refuses liquids, 1 = refuses solids, 2 = decreased, and 3 = normal), and motor function (0 = unable to stand, 1 = unable to walk, 2 = unsteady gait, and 3 = normal). Numeric summing of these functions provides a final score, with the maximum (score of 9) reflecting apparently normal neurologic function, and lower values indicating substantial brain damage.

Perfusion Fixation

Each surviving animal was electively killed on the seventh postoperative day. Immediately after intravenous injection of pentobarbital (60 mg/kg) and heparin (500 IU/kg), the thoracic cavity was opened, and the descending thoracic aorta was clamped. Ringer solution (1 L) was infused through the ascending thoracic aorta and through the upper body, and blood was suctioned from the superior vena cava until the perfusate was clear of blood. Then 10% formalin solution (1 L/15 min) was perfused through the brain in the same manner to accomplish a perfusion fixation. Immediately thereafter, the entire brain was harvested, weighed, and immersed in 10% neutral formalin. The same method of fixation procedure was carried out in those animals that died before the seventh postoperative day.

Histopathologic Analysis

The brain was allowed to fix for 1 week en bloc. Thereafter, 3-mm-thick coronal samples were sliced from the left frontal lobe, thalamus (including the adjacent cortex), and hippocampus (including the adjacent brainstem and temporal cortex), and sagittal samples from the posterior brainstem (medulla oblongata and pons) and cerebellum were obtained. The specimens were fixed in fresh formalin for another week. After fixation, the samples were processed as follows: rinsing in water for 20 minutes and immersion in 70% ethanol for 2 hours, 94% ethanol for 4 hours, and

absolute ethanol for 9 hours. Then the specimens were kept for 1 hour in an absolute ethanol-xylene mixture and for 4 hours in xylene and embedded in warm paraffin for 6 hours. The specimens were sectioned at 6 μm and stained with hematoxylin and eosin. The sections of the brain specimens of each animal were screened by an experienced senior pathologist (J.H.) unaware of the experimental design and the identity and fate of individual animals. Each section was carefully examined for the presence or absence of any ischemic or other kinds of tissue damage.

The signs of injury were scored as follows: 1 (slight edema, with dark or eosinophilic neurons or cerebellar Purkinje cells); 2 (moderate edema, with at least 2 hemorrhagic foci in the section); and 3 (severe edema, with several hemorrhagic foci and infarct foci [local necrosis]). The total regional score was the sum of the scores in each specific brain area (cortex, thalamus, hippocampus, posterior brainstem, and cerebellum). A total histopathologic score was calculated by summing all the regional scores to allow semiquantitative comparisons between the animals.

Evaluation of Apoptosis

Brain tissue specimens were further evaluated for the extent of apoptosis by means of 3′ end-labeling of apoptotic DNA (TUNEL procedure) with the ApopTag in situ Apoptosis Detection Kit (Oncor, Gaithersburg, Md). A cell was defined as apoptotic if the whole nuclear area showed a positive brown reaction, whereas apoptotic bodies appeared as small positively labeled globular fragments. All positively labeled cells and bodies showed the morphologic criteria for apoptosis (ie, nuclear condensation, cell shrinkage, cytoplasmic budding to form membrane-bound fragments, and detachment from surrounding cells). The number of apoptotic cells and bodies within the infarct area was counted in at least 5 high-power fields (magnification 40×). The quantity of apoptotic cells and bodies (ie, apoptotic index [AI%]) was expressed as a percentage of the whole cell population within the infarct area.

Statistical Analysis

Statistical analysis was performed with SPSS software (SPSS version 10.0; SPSS Inc, Chicago, Ill). Values are expressed as the median with interquartile ranges (25th-75th percentile). Difference in survival outcome in the study groups was evaluated with the Fisher exact test. Differences between the groups were determined by using the *t* test or the Mann-Whitney test. Repeated-measures analysis of variance was used, and the results of the tests of between-subject effects were reported.

Results

Comparability of the Groups

The median weight of the pigs was 27.2 kg (interquartile range, 25.8-29.5 kg) in the EPO group and 26.1 kg (interquartile range, 25.0-28.7 kg) in the control group ($P = .49$). The median CPB cooling time was 60.0 minutes (interquartile range, 60.0-61.0 minutes) in the EPO group and 60.5 minutes (interquartile range, 60.0-61.5 minutes) in the control group ($P = .64$). The median CPB rewarming time was 63.5 minutes (interquartile range, 61.8-68.0 minutes) in the EPO group and 62.5 minutes (interquartile range, 60.8-64.8 minutes) in the control group ($P = .24$). The median total CPB time was 123.5 minutes (interquartile range, 122.0-128.0 minutes) in the EPO group and 124.0 minutes (interquartile range, 121.0-127.0 minutes) in the control group ($P = .39$). During the experiment, rectal, esophageal, epidural, and cerebral temperatures did not differ between the groups (the latter is shown in Figure 1).

Plasma and Cerebrospinal Fluid EPO Concentrations

The median baseline EPO concentration in plasma was 20.1 U/L (17 and 23 U/L, n = 2). After the intravenous injection of r-Hu-EPO, the median EPO concentrations in plasma were 1397 U/L (range, 1300-1456 U/L) at the end of cooling (n = 3), 1422 U/L (1378 U/L and 1465 U/L) 40 minutes after the start of rewarming (n = 2), 1541 U/L (1436 U/L and 1646 U/L) 8 hours after the start of rewarming (n = 2), and 1068 U/L at extubation (n = 1).

The median baseline EPO concentration in the cerebrospinal fluid was 8.2 U/L (range, 7.2-9.8 U/L) in the EPO group (n = 5) and 7.7 U/L (range, 7.2-9.1 U/L) in the control group (n = 5). Eight hours after the start of rewarming, the concentration was 36.7 U/L (range, 34.7-92.9 U/L) in the EPO group (n = 3) and 8.4 U/L (range, 6.7-9.8 U/L) in the control group (n = 4). At extubation, the concentration was 14.5 U/L (11.5 U/L and 17.5 U/L) in the EPO group (n = 2) and 8.4 U/L (range, 7.4-8.8 U/L) in the control group (n = 3).

Mortality and Behavioral Outcome

The 7-day survival rate was 60% in the EPO group and 70% in the control group ($P = 1.0$). There were no significant differences between the study groups in the overall postoperative behavioral scores ($P = .85$, Figure 2). Among those animals that survived all 7 days, the scores were similar between the groups ($P = .53$).

Hemodynamic and Metabolic Data

There were some differences between the study groups in terms of hemodynamics (Figure 3). Vascular resistance was higher in the EPO group from the fourth hour after the start of rewarming until extubation. The EPO group had significantly higher mean arterial pressure values ($P = .04$, tests of between-subjects effects) throughout the experiment. The cardiac index tended to be lower in the EPO group during the postoperative period, which led to a significantly lower oxygen delivery rate at extubation ($P = .04$). However, there were no significant differences between the groups in the amount of dopamine administered (data not shown).

The study groups did not differ significantly in terms of pH, oxygen and carbon dioxide tension, mixed venous oxygen saturation, oxygen consumption, total leukocyte and neutrophil counts, and venous concentrations of glucose, lactate, sodium, and potassium (data not shown).

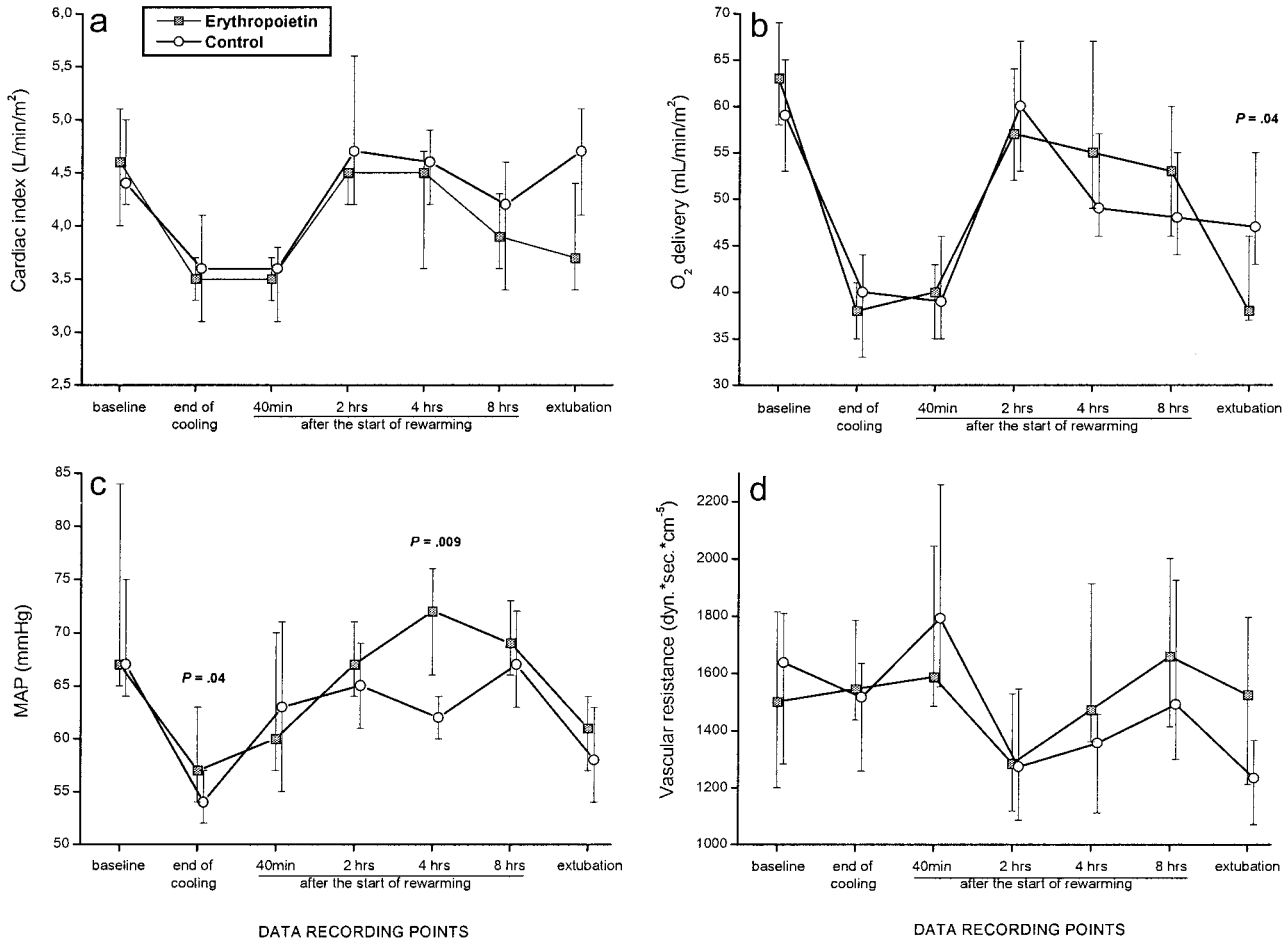

Figure 3. Hemodynamic parameters. Values are expressed as medians with interquartile ranges (25th-75th percentile). *P* values are for the EPO group versus the control group. *MAP,* Mean arterial pressure (in this parameter, *P* value between groups is .04, according to the tests of between-subjects effects).

Measurements of serum concentrations of total CK showed that this parameter tended to be higher from the end of cooling until the fourth hour after the start of rewarming among pigs that received EPO. The difference in total CK levels was related to the difference in CK-MM levels because the concentrations of CK-MB and CK-BB were similar between the study groups (data not shown).

Intracranial Measurements

Intracranial measurement data are presented in Figure 4. Intracranial microdialysis showed some differences between the study groups. Brain glucose tended to be higher in the control group from the 5-hour until the 12-hour interval after the start of rewarming. Brain glutamate was higher in the control group throughout the postoperative period. This difference was statistically significant at 6-hour ($P = .03$) and 7-hour ($P = .03$) intervals after the start of rewarming. Also, brain glycerol tended to be higher in the control group during the first postoperative hours. However, brain lactate levels did not differ between the study groups.

Brain tissue oxygen partial pressure tended to be higher in the EPO group during the last hours of the experiment. Intracranial pressure levels were similar in both groups throughout the experiment.

Electroencephalographic Findings

The electroencephalographic findings before and after the intravenous administration of r-Hu-EPO were similar, thus suggesting that the drug did not have any effect on electroencephalographic activity. The median rate of electroencephalographic burst recovery from the start of rewarming until extubation was similar in both groups. At the 7-hour interval after the start of rewarming, the median electroencephalographic burst-suppression ratios were close to 100% in both groups (ie, electroencephalography was continuous and without suppressions).

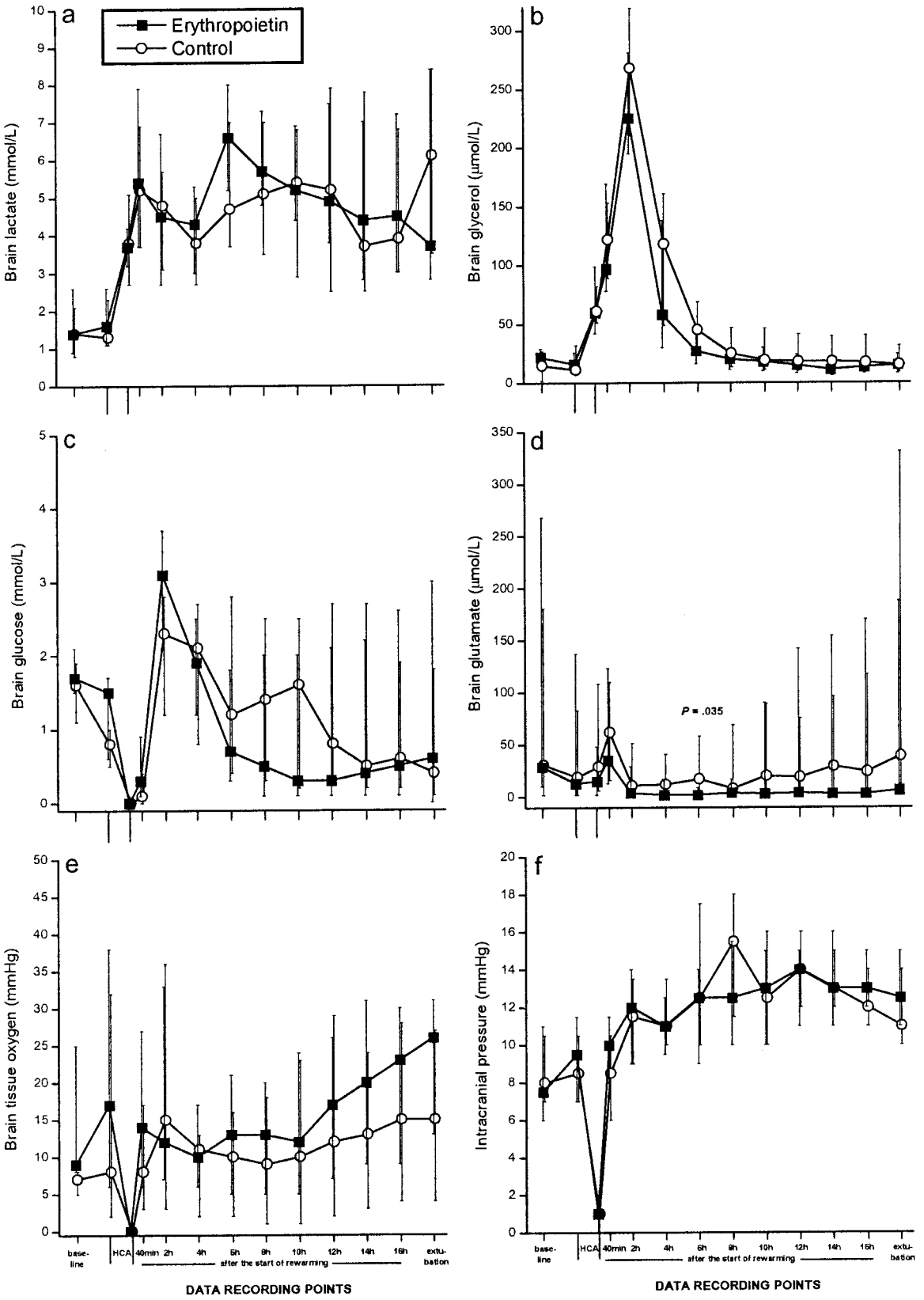

Figure 4. Results of brain microdialysis, intracranial pressure, and brain tissue oxygen partial pressure measurements. Values are expressed as medians with interquartile ranges (25th-75th percentile). *P* values are for the EPO group versus the control group.

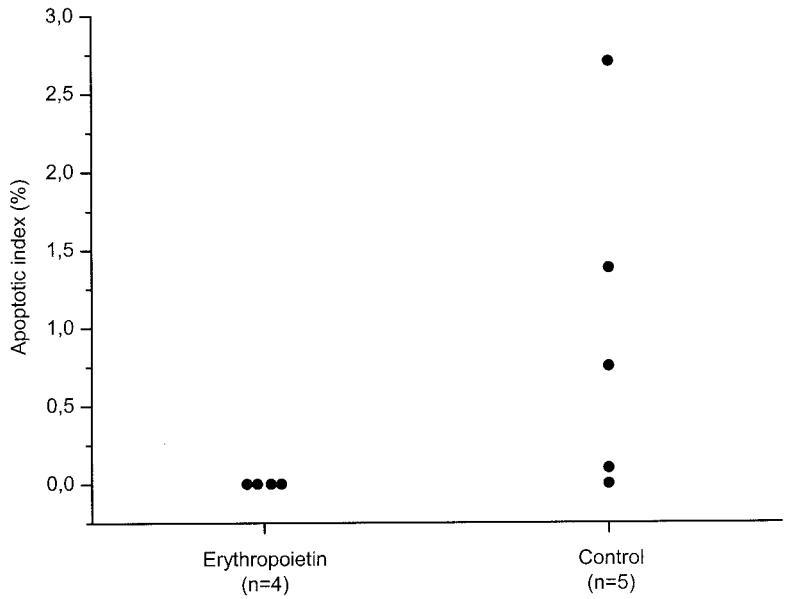

Figure 5. Brain cellular apoptotic indexes in pigs surviving for 7 days that underwent brain infarction ($P = .06$, EPO group vs control group).

TABLE 1. Histopathologic scores

Protocol	Pig no.	Survival (day)	Cortex score	Thalamus score	Hippocampus score	Posterior brainstem score	Cerebellum score	Total score
EPO	1	7	5	1	2	1	1	10
	2	7	5	1	2	1	0	9
	3	7	2	1	2	1	1	7
	4	1	5	4	1	3	3	16
	5	1	6	2	1	4	4	17
	6	1	6	5	3	4	4	22
	7	7	5	1	1	1	1	9
	8	1	5	4	1	1	1	12
	9	7	2	1	1	1	0	5
	10	7	2	1	2	1	0	6
Mean		**4.6**	**4.3**	**2.1**	**1.6**	**1.8**	**1.5**	**11.3**
Control	1	7	5	2	2	3	2	14
	2	7	5	1	2	0	0	8
	3	7	7	1	2	1	1	12
	4	7	8	2	4	1	1	16
	5	1	2	1	1	1	1	6
	6	2	4	4	3	3	4	18
	7	7	1	0	1	0	1	3
	8	7	2	1	2	1	1	7
	9	7	2	1	1	1	0	5
	10	1	6	3	3	4	4	20
Mean		**5.3**	**4.2**	**1.6**	**2.1**	**1.5**	**1.5**	**10.9**

Signs of brain ischemic injury were scored as follows: 1 (slight edema, with dark or eosinophilic neurons or cerebellar Purkinje cells); 2 (moderate edema, with at least 2 hemorrhagic foci in the section); and 3 (severe edema, with several hemorrhagic foci and infarct foci [local necrosis]). The total score is the sum of the scores for each specific brain area.

Histopathologic Data

The total histopathologic score among the animals of the EPO group did not differ significantly from those of control animals (11.3 vs 10.9, respectively; $P = .8$). Also, the scores from different brain regions (cortex, thalamus, hippocampus, posterior brainstem, and cerebellum) did not differ significantly between the groups (Table 1).

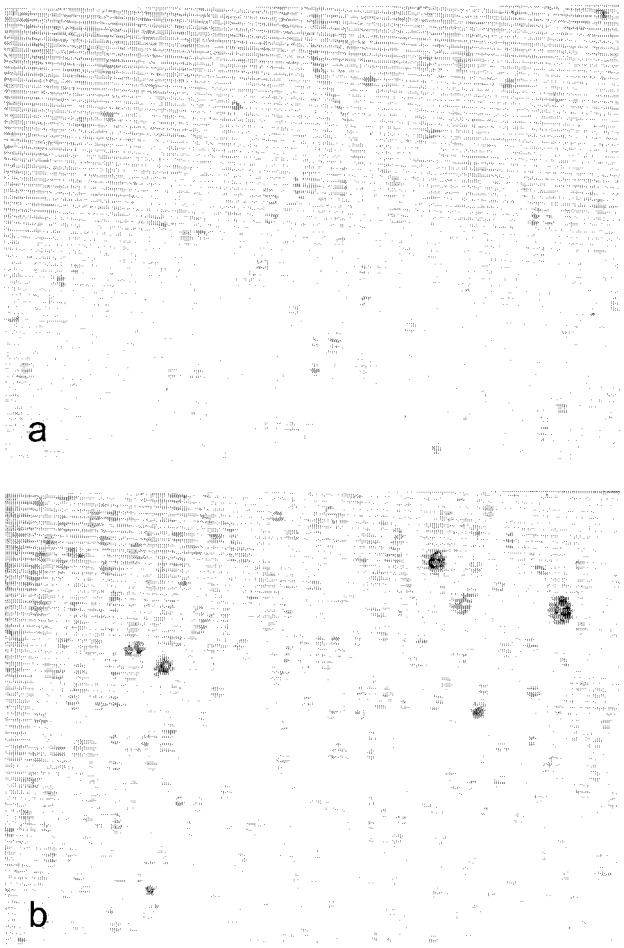

Figure 6. Brain tissue sections after 3′ end-labeling of apoptotic DNA (TUNEL) of an animal that received r-Hu-EPO preoperatively (a) and a control animal (b). (Original magnification 40×.) No apoptotic staining is present in the EPO-treated animal, whereas several brown apoptotic cells can be seen in the control animal.

Apoptosis

Thirteen pigs survived 7 days. Among these pigs, there were 4 animals in the EPO group and 5 in the control group that had brain infarction. The mean apoptotic index was 0 in the EPO group and 0.99 in the control group ($P = .06$, Figures 5 and 6).

Discussion

Recent studies demonstrated that EPO has several biologic activities other than the hematopoietic one. EPO mRNA and receptors have been found in brain tissue, and the neuroprotective effect of r-Hu-EPO has been demonstrated in both in vitro and in vivo stroke models, as well in brain injury after trauma, subarachnoid hemorrhage, inflammation, and seizures.[10,11] EPO is likely to be produced by astrocytes in a hypoxia-inducible manner, whereas exogenous EPO could reach the neurons only through a damaged blood-brain barrier.[4,12] However, Brines and colleagues[1] observed that EPO and its receptors are abundantly expressed in brain capillaries, thus probably providing a route for circulating EPO to enter the brain. They also observed that biotinylated r-Hu-EPO crosses the blood-brain barrier and that intraperitoneal injection of r-Hu-EPO was associated with an increase in EPO concentrations in the cerebrospinal fluid.

The mechanisms underlying the neuroprotective effects of EPO are not clear. EPO can rescue cells from death through modulation of apoptosis,[5,8,13,14] necrosis, inflammatory injury,[1,8] or neuronal excitability.[1] It has been suggested that EPO contributes to nitric oxide metabolism and activates Ca^{2+} channels[2,5,7,15] and that EPO has a specific neurotrophic activity.[8,16]

The present study did not show any striking neuroprotective effects of EPO in an experimental model of HCA. However, brain glutamate and glycerol levels and brain tissue oxygen tension tended to be more favorable in the EPO group, thus suggesting that EPO might have some neuroprotective effects after global brain ischemic injury. Interestingly, the apoptotic activity in the infarct area was lower among the EPO-treated animals 7 days after the ischemic event. This finding mirrored other observations on EPO-induced inhibition of apoptosis.[5,8,13,14] Siren and coworkers[8] suggested that EPO inhibits the ischemia-induced neuronal cell apoptosis through activation of the bcl family of antiapoptotic genes, thus activating the specific protein kinases to promote cell survival.

The results of the present study could have been affected by the timing of EPO administration. Calapai and associates[7] observed in their stroke model that systemic pretreatment with r-Hu-EPO 60 minutes before ischemia failed to improve brain ischemic injury, whereas the administration of the drug immediately after the ischemia produced beneficial effects. However, Brines and colleagues[1] demonstrated a similar effect of neuroprotection between preischemic and postischemic administration of r-Hu-EPO.

It is possible that the EPO concentrations achieved in the cerebrospinal fluid after a single intravenous dose are not enough to provide adequate brain protection in the present animal model of HCA. Daily administration of EPO during the 7 postoperative days would have probably strengthened the possible neuroprotective effect, hypothetically by means of a further reduction of cellular apoptosis.

The use of EPO in this study was associated with an increase in vascular resistance, which resulted in higher mean arterial pressure values, as well as in a decreased cardiac index and oxygen delivery rate. An increase of hematocrit,[17] endothelin production,[18] and tissue renin-angiotensin activity,[19] as well as a direct vasopressor action of EPO,[20] have been suggested to be implicated in the pathogenesis of hypertension during long-term administra-

tion of EPO. It is possible that, in the present study, a direct vasoconstrictive effect of EPO could have also affected the cerebral blood flow, and this might have masked the possible neuroprotective effect of the drug.

In conclusion, the administration of 500 IU/kg r-Hu-EPO intravenously before experimental HCA was followed by a long-lasting increase of EPO concentration in the cerebrospinal fluid. Although in vitro and in vivo stroke model studies have demonstrated neuroprotective effects of EPO during brain ischemia, the present study, using a chronic porcine model of HCA, failed to show any significant benefit after administration of r-Hu-EPO in terms of mortality, electroencephalographic findings, and brain histopathologic findings. Lower intracerebral concentrations of glutamate and glycerol, higher brain tissue oxygen partial pressure, and lower apoptotic index observed among the EPO-treated animals, however, suggest that a distinct neuroprotective effect of r-Hu-EPO might be achieved at different dosages and timing of administration.

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